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Original Article

Interleukin 17 Cytokine Profiles in Patients with Cystic Echinococcosis in Babylon Province, Iraq

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Abstract

Cystic Echinococcosis (CE) is the silent chronic Helminthes zoonotic infection caused by the larval stage in intermediate hosts of the dog tapeworm *Echicoccous granulosus*, which belongs to the Taeniidae family and genus *Echinococcu* ssp. According to the study of CE, the hospitalization and surgeries of patients indicated the high prevalence of the disease in Iraq.This study aimed to determine some immunological parameters in patients infected with *Echinococcus granulose.* The study of 53 patients infected with CE showed that in 40 (75.4 %), 5 (9.4%), 4 (7.5%), and 2 (3.7 %) cases the liver, abdominal cavity, kidney, and lungs were involved. In terms of age, most and least number of the patients were within the age ranges of30-40 (n=15, 28.3 %) and60-70 years old (n=7, 13.2 %), respectively. Moreover, 37(56.9%) and 16(43%) of them were female and male, respectively. The enzyme-linked immunosorbent assay (ELISA) was used to measure the level of the Interleukin family among patients. There was a significant increase in the serum level concentration of IL17A and IL17B in patients with hydatid disease, compared to the control group. The changes in different age groups also showed statistically significant differences among them ($P \leq 0.05$). The outcome of this study indicated that CE is endemic in Babylon province, Iraq. The ELISA technique is a reliable and efficient test for the early diagnosis and monitoring of human hydatid disease. Moreover, it was found that the liver was the most common site of human hydatid cyst.

Keywords: Echinocooccosis, *Echinococccus granuulosus*, Interleukin17, Iraq

1. Introduction

Echinococcosis is an important chronic helminth zoonotic infection caused by the larval stage (cestode) of tapeworm *Echicoccous granulosus* in humans as well as domestic and wild mammals that belong to the Taeniidae family (1). The two-major species of *Echinococcus* of medical and public health importance are *E. granulosus* and *E. multilocularis*, which cause cystic echinococcosis (CE) and alveolar echinococcosis in humans, respectively (2). The disease can be life-threatening in humans, causes important public health problems, and has a significant economic impact on livestock producers (3). Although CE is typically asymptomatic in affected livestock, the organs affected with cysts, which are detected on inspection at slaughter, are usually totally condemned resulting in financial loss for the producers. Severe cases in livestock may result in reduced productivity due to interference with organ function (4).

In Iraq, human CE is endemic, and the condition has been identified based on the number of individuals hospitalized and surgically treated (5). The intermediate host immunity plays an important role in determining the relationship between the host and the parasite. Parasite produces excretory compounds, which influence the immune-competent cells in the human

host and stimulate pro-inflammatory immune responses, releasing antibodies and activating T-cells in the body (6).

Cytokines are small secreted proteins released by cells and have a specific effect on the interactions and communications between cells (7). The IL-17A is a key pro-inflammatory cytokine in the T helper 17 pathway and plays a critical role in host defense and inflammation. Some animal studies have revealed that helminthic infections improved the severity of autoimmune diseases by reducing the Th17 response (8). TheIL-17B was originally identified as a proinflammatory mediator that accelerated neutrophil recruitment and migration (9). Moreover, theIL-17B inhibited IL-25 signaling and attenuated mucosal inflammation. Although low IL-17B mRNA is detected in several organs, its expression is high in chondrocytes and neurons (10).

The role of IL-17 cytokines and Th17-type immune responses in CE disease is yet unexplored. The six identified family members (IL17A-F) exert mostly pro-inflammatory activities .The IL17A and IL17F, mediators of the recently described proinflammatory Th17-type immune responses have been associated with inflammatory disorders, like rheumatoid arthritis and inflammatory bowel disease (11). Epidemiological studies of the disease in recent years have shown that human CE is endemic in some parts of Iraq. Analysis of the levels of pro-inflammatory IL-17 members (IL-17A, IL-17B) in clinically staged CE patients is one of the ways to identify the immune response, which can help control and prevent the disease.

2. Materials and Methods

2.1. Study Population

This study was performed on patients with hydatid cyst, referred to Babylon Teaching Hospital, Imam Al-Sadiq Hospital, and private Hospitals in Babylon province, Iraq. All samples were collected from 2019 to 2020. In this study, a total of 93 people were evaluated in two groups of case (n=53) and control with no clinical sign (n=40). The participants were within the age range of 20-70 years. The practical part of the study was performed at the microbiology laboratory at the College of Medicine, University of Babylon, Babylon, Iraq. For the study of sociodemographic and associated factors in the epidemiology and prevalence of CE, various factors, such as age, gender, location (urban or rural), and organs involved in the disease were evaluated.

2.2. Sample Collection

The blood samples were collected from all individuals by using a disposable syringe (5 ml). In total, 5 mL of blood was obtained from each patient and then pushed into two tubes (2.5 mL blood in the ethylenediaminetetraacetic acid tube and 2.5 ml blood in the gel tube).The gel tube of blood was centrifuged at 14,000 rpm for approximately 10-15 min; afterward, the serum was stored at -20˚C until analysis.

2.3. Enzyme-Linked Immunosorbent Assay

The analysis of the IL-17 family (IL17 A, B, RA, and RB) in patients and controls was performed using the enzyme-linked immunosorbent assay (ELISA) test (the procedure was achieved according to the method recommended by the manufacturing company, Bioassay technology, China) according to the instructions of the manufacturer. The ELISA plates were coated with the capture antibody specific for the IL-17 family (IL17 A, B, RA, and RB) and cytokines.

For each cytokine, the ELISA test was performed as follows: the plate was pre-coated with human IL-17A, B, RA, and RB antibodies. The IL-17 family present in the sample was added and bound to antibodies coated on the wells. Next, the biotinylated human IL-17A antibody was added and bound to the IL-17 family in the sample. Afterward, Streptavidin-horseradish peroxidase was added and bound to the Biotinylated IL-17A antibody. After incubation, unbound Streptavidin-horseradish peroxidase was washed away during a washing step. Substrate solution was then added and color was developed in proportion to the amount of human IL-17A. The reaction was terminated by the addition of acidic stop solution and absorbance was measured at 450 nm.

2.4. Statistical Analysis

The experiments were conducted in duplicate, and quantitative data were described as mean±SD, and the results were analyzed in SPSS software (version 23). The level of statistical significance was set at alpha equal to 0.05 (P=0.05). A p-value of less than 0.05 was considered statistically significant, and a p-value of less than 0.01 was considered highly statistically significant (12).

3. Results and Discussion

3.1. Sociodemographic and Associated Factors with Cystic Echinococcosis

Among the 53 CE patients, 37(56.9%) and 16(43%) were female and male, respectively, as shown in figure 1. Moreover, 13(24.5%) and 40 (75%) cases were from urban and rural areas of Babylon province, respectively (Table 1). In 40 (75.4 %), 5 (9.4%), 4 (7.5%), and 2 (3.7 %) cases the liver, abdominal cavity, kidney, and lungs were involved (Table 2). In terms of age, it was observed that the maximum and minimum number of patients were within the age ranges of 30-40 (n=15, 28.3 %) and 60-70 years (n=7, 13.2 %) respectively (Table 3).

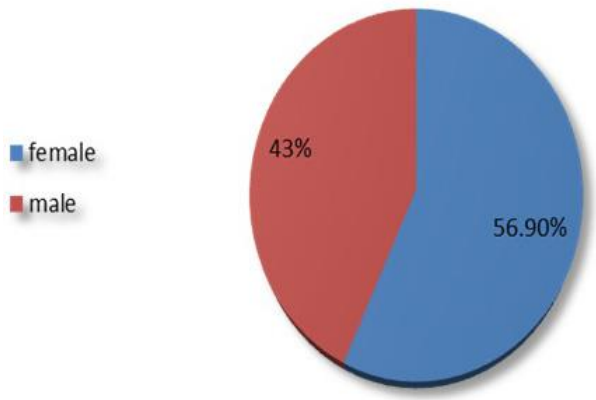

Figure 1. Gender distribution among hydatid cyst patients

According to figure 1, the rate of CE was significantly higher in females, compared to males, which was in line with the findings of other studies (13). Perhaps this is due to the fact that females are more commonly involved in dealing with meat, contaminated food and vegetables, and animals (14). However, investigational studies have shown that the males were more prone to contracting the disease than the females (15). Furthermore, according to table 1, in terms of residence, the infection was more prevalent in rural areas, compared to urban areas.

Table 1. Distribution of *Echicoccous granulosus* patients according to the residence (urban vs. rural)

Residence	*Echicoccous granulosus* infection	
	No. of patients	Percentage (%)
Urban	13	24.5
rural	40	75
Total	53	100

This result was in line with those of the study performed by Saida and Nouraddin (16) on 149 patients with cystic hydatidosis which showed that 82 (55.03%) and 67 (44.97%) of them lived in rural and urban areas, respectively. This result may be due to many factors, including poor living conditions, lack of adequate health education in rural areas, economic instability, and financial restrictions in terms of control and prevention. This result was in line with those of many previous studies (17) which have indicated that the infection rate is higher in rural areas, compared to urban areas.

However, this was inconsistent with the results of the study conducted by Ahmed, Mero (18) which showed that the infection was more prevalent among urban dwellers(87%), compared to rural dwellers (13%) (P<0.001). Findings of another investigation (19) revealed that among18 patients with human hydatid cyst samples, the rate of infection was higher among those who lived in urban areas, compared to rural areas, which was inconsistent with the findings of the current study. This was not optimal since both studies were conducted in rural/urban areas, and it appears that CE is being urbanized and can no longer be considered solely a rural disease (20).

As for the organs involved in the disease, it is shown in table 2. These results of the current study were inconsistent with those of the research performed by Ahmed, Mero (18) who confirmed that the majority of human infections were in the lung (58.82%), while the liver had the lowest rate in this regard (41.18%). However, results of the study carried out by Saida and Nouraddin (16) indicated that the liver had the highest rate of involvement (n=31, 55.3%) followed by lungs (n=9, 16.07%) in Sulaymaniyah Province, Iraq.

Results of the present study were in line with those of the study conducted by Al-Saeed and Al-Mufty (21) which showed that the liver was the most common site of hydatid disease in 26 (54.2%) cases. The liver acts as the primary filter for the parasite and the lungs act as the secondary filter. However, in few studies, some researchers have found that the lungs were the predominant site of hydatid disease. During the years 1986-1990 in Iran, 4.850 cases of hydatid disease were operated on, 46.2% of which were lung cysts while 42% of them were liver cysts (22).

Table 2. Distribution of *Echicoccous granulosus* according to the organ infection

Organ	Number of cystic echinococcosis cases	Percentage	Gender(male/female)
Liver	40	75.4	10/30 case
Lungs	2	3.7	2/0 case
Abdominal cavity	5	9.4	1/4 case
Kidney	4	7.5	1/3 case
Brain	2	3.7	2/0 case
Total	53	100	53

In this study, as usual, in hydatid infection, the liver in both genders was more frequently involved than the other organs followed by the abdominal cavity. These findings were in line with those of other studies (16). In many cases, the resistances shown by the liver tissue surrounding the cyst slowed or even prevented their growth for many years. On the other hand, the lungs show lower resistance to the growth of the hydatid cyst due to their elasticity, and this state allows an increase in the cyst size (23).

Table 3 shows the age distribution of patients with hydatid disease; it was found the highest prevalence rate was within the age range of 30-40 (28.3%) years old and also the results showed that the lower infection occurred in 41-51 years old individuals.

Moreover, results of the present study were in line with those obtained by Obaid (24) during his study of infected people in Kirkuk, Iraq where he recorded the highest rate (40%) of infection in CE male patients within the age range of 56-64 years followed by those who were 20-28 years old (18.2%).

Table 3. Distribution of *Echicoccous granulosus* according to based on age groups

Age (years)	Number of cystic echinococcosis cases	Percentage	Gender (male/female)
20-30	12	22.6	4/8 cases
30-40	15	28.3	3/12cases
41-50	8	15.09	3/5cases
51-60	11	20.75	4/7cases
60-70	7	13.2	2/5cases
Total	53	99.9	53 cases

3.2. IL-17 Levels in a Patient with Cystic Echinococcosis

The IL-17 family rate of CE patients in responses to infection was evaluated using the ELISA test. As shown in table 4, serum IL-17 levels were slightly higher in patients with CE. Moreover, based on the findings, levels of IL17A in the age group of >40 years underwent an increase by 241.51±29.3 pm/ml, compared to the controls (increase by 133.6±26.8 pm/ml). These results were significantly in line with those of a study performed by Mezioug and Touil-Boukoffa (25). Interleukin-17A (IL-17A) production in patients with CE and the role of IL-17A in the modulation of the immune response against the extracellular parasite were examined in a previous study. In the aforementioned study, in the *E. granulosus*, Cytokine production was also measured from hydatid patients stimulated by a major parasitic antigen (antigen-5); furthermore, the activity of IL-17A increased in most serum samples from patients (26). In contrast, healthy controls showed only minor levels, and this was in line with the results of the present study which showed that IL-17A was produced during human CE and was involved in the host defense mechanisms against the extracellular parasite *E. granulosus*. This suggests that IL-17A plays an immune protective role in this parasitic helminth infection (27).

Table 4. Concentration levels of IL17A based on the age of patients and controls

Parameter	Age(years)		Concentration pg/ml Mean±SD	p-value
IL17A	20-30	Patient	224.31±68.7	0.03
		Control	186.9±13.5	
	31-40	Patient	238.20±34.1	0.01**
		Control	156.7±35.8	
	41-50	Patient	241.51±29.3	0.02
		Control	133.6±26.8	
	51-60	Patient	244.39±34.6	0.2
		Control	135.6±23.5	
		Patient	251.78± 25.3	0.00**
		Control	129.4±33.1	

**significant at≤ 0.05

The results showed that the levels of IL-17B significantly increased in all CE patients in comparison to the healthy controls (Table 5). There was a significant difference between the CE patients in the case group; accordingly, lower concentrations of IL-17B were detected in cured cases of CE, while higher concentrations were observed in progressive cases.

Moreover, IL-17B was 31.59±4.3 pg/mL in the age range of 20-30 years detected in control cases was (16.9±5.1) pg/mL of CE, while highest concentrations were observed in (61-70) age (36.68± 11.5) pg/ mL of progressive cases.

In turn, other family members derive from different cellular sources and are associated with varying functions. The IL-17A, IL-17F, IL-17C, and IL-17B function in host defense against pathogens and play various but not fully understood roles in mediating inflammation in autoimmune, allergic, and chronic

inflammatory conditions (28).

Plenty of evidence suggested that cytokines play a crucial role in the immune response process as both Th1 and Th2 cytokines during hydatid disease (29). Published data indicated that Th1 cytokines are related to protective immunity, whereas Th2 cytokines are associated with the chronic stage, clinical complications, and secondary episodes (30). The role of IL-17B in host defense against intracellular protozoan parasites remains less well studied (31).

Table 5. Concentration levels of IL17B based on the age of patients and controls.

Parameter	Age (years)		Concentration pg/ml Mean±SD	p-value
IL17B	20-30	Patient	31.59±4.3	0.4
		control	16.9±5.1	
	31-40	Patient	35.84±10.2	0.00**
		control	19.6±6.4	
	41-50	Patient	34.69±5.01	0.01*
		control	19.1±4.6	
	51-60	Patient	33.62± 3.1	0.06
		control	16.8±6.1	
	61-70	Patient	36.68± 11.5	0.1
		control	17.9±3.8	

*,**significant at≤ 0.05,

3.3. Correlation between IL-17A and IL17B

There was a positive correlation between IL-17A and IL 17 B by a recorded p-value of 0.01 in patients with CE (Figure 2). Moreover, there was a significant difference between IL17A and IL17RA.

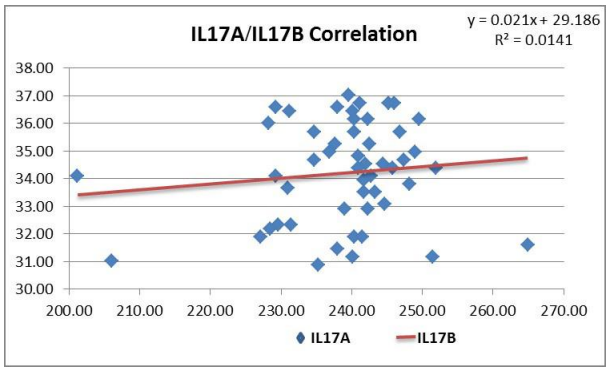

Figure 2. Correlation between IL-17Awith IL17B in patients of Cystic Echinococcosis

The cytokines IL-17A and IL-17 F are highly homologous and bind to the same receptor, and both contribute similarly to the progress of inflammation and the host defense against pathogens (32). Several studies have described the importance of cytokines and chemokines in the prevention and elimination of parasite infection, with the tapeworm *Echinococcussp.* infections. The Th1-type cytokine IL-12 and IFN-gamma have been identified to successfully kill the larval stages of the parasite (metacestode) at the initial stages of development, whereas Th2 immune responses induced by IL-4, IL-5, IL17 family, their receptor, and IL-10 lead to a chronic course of disease (33).

The results of this study indicated that hydatidosis is endemic in Babylon province. In addition, it was revealed that the ELISA technique is reliable and efficient for the early diagnosis and monitoring of human hydatid disease. Moreover, it was found that the liver was the most common site of human HC (65.3%) followed by the abdominal cavity (9.4%), and lungs (3.7%).

Authors' Contribution

Study concept and design: H. K. A.
Acquisition of data: K. C. A.

Analysis and interpretation of data: I. A. K.
Drafting of the manuscript: H. K. A.
Critical revision of the manuscript for important intellectual content: K. C. A.
Statistical analysis: I. A. K.
Administrative, technical, and material support: H. K. A.

Ethics

All procedures performed in this study involving human participants were in accordance with the ethical standards of the University of Babylon, Baghdad, Iraq under the project number of 2019-5475-5745.

Conflict of Interest

The authors declare that they have no conflict of interest.

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